APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2004; **18**: 589–594
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.744

Why do nitrogenases waste electrons by evolving dihydrogen?†

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Received 5 April 2004; Accepted 5 April 2004

In nature, nitrogen is commonly fixed as the most reduced form, ammonia (NH_3) and as the most oxidized form, i.e. nitrate ion (NO_3^-). Nitrogenases catalyze the reduction of N_2 into NH_3 by using protons and electrons with evolution of H_2. However, the reason why the enzymes waste electrons by evolving H_2 has yet to be clarified. We have previously reported (*J. Am. Chem. Soc.* 2002; 124: 597) pH-dependent heterolytic cleavage of H_2 and subsequent reduction of NO_3^- with evolution of H_2 by iridium complexes in water. We propose herein a catalytic mechanism of nitrogenases, which can account for evolution of H_2 in the reduction of N_2 to NH_3 in relation to a mechanism of the reduction of NO_3^-. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: hydrogen evolution; hydride; nitrogen fixation; nitrogenase

REDUCTION OF N_2 BY NITROGENASES WITH EVOLUTION OF H_2

Nitrogenases are bacterial enzymes that catalyze the reduction of dinitrogen (N_2) to ammonia (NH_3) by using protons and electrons at ambient temperature and pressure in the presence of water.[1–9] X-ray crystallographic studies have shown that the active site (FeMoco) of the enzymes consists essentially of two incomplete cubane clusters, one based on the Fe_4S_3 composition and the other based on the $MoFe_3S_3$ composition, bridged by three μ_2-sulfide ligands (Fig. 1).[10–24] The active site is bound to the polypeptide via Cys275 to the tetrahedral ion and His442 to the octahedral molybdenum. Homocitrate is also bound to the molybdenum as a bidentate ligand.

The reduction of N_2 to NH_3 by nitrogenases accompanies futile evolution of H_2:[1,25,26]

$$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2 \quad (1)$$

An important question arises here: Why do the enzymes waste the electrons as H_2 in order to reduce N_2 to NH_3? Figure 2 shows H_2-evolution pathways in the nitrogenase reaction cycle proposed by Thorneley and Lowe.[27–31] The reduced states of MoFe–protein are represented by E_n. The subscript n is the total number of electrons transferred to the MoFe–protein. In the various states, only E_3H_3 and E_4H_4 can bind N_2 with evolution of H_2. Based on these enzymatic studies, it has been proposed that E_3H_3 is a metal dihydride species represented by $E_3H_2(H^+)$, which is activated by protonation from an adjacent amino acid side chain (Fig. 3, where M is the metal ion and the superscript n is the oxidation number).[1,32] In this mechanism, H_2-evolution occurs by the reaction of the hydride ligand with the proton bound to the adjacent amino acid. In such a case, the resulting species (M^n–H) has a vacant coordination site at which N_2 can be bound, although the oxidation number of the metal ion remains the same after the evolution of H_2.

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Contract/grant sponsor: Ministry of Education, Science, Sports, and Culture.
Contract/grant sponsor: Japan Society for the Promotion of Science.
Contract/grant sponsor: Grant-in-Aid for Scientific Research; Contract/grant number: 11228205; 15036242; 15350033.
†Presented in part at the XVth FECHEM Conference on Organometallic Chemistry, held 10–15 August 2003, Zürich, Switzerland.

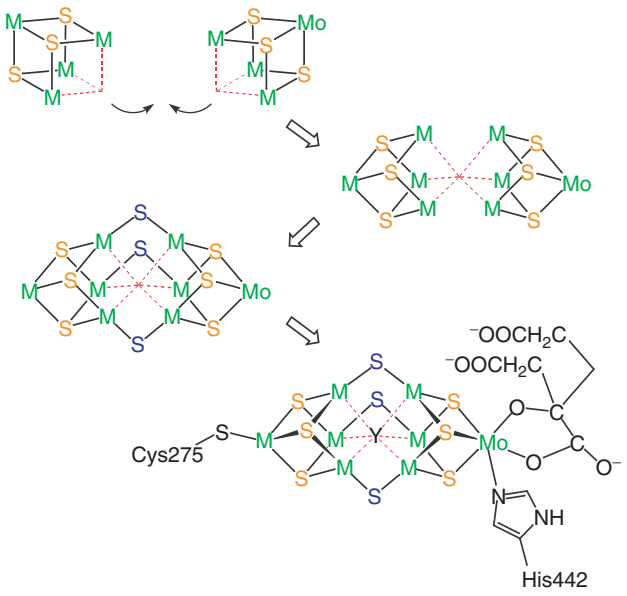


Figure 1. A concept of building-block synthesis of the active site in MoFe–nitrogenases (M = Fe, S = μ_2-sulfide ligand, S = μ_3-sulfide ligand, Y = an unrecognized coordination site).[10–15]

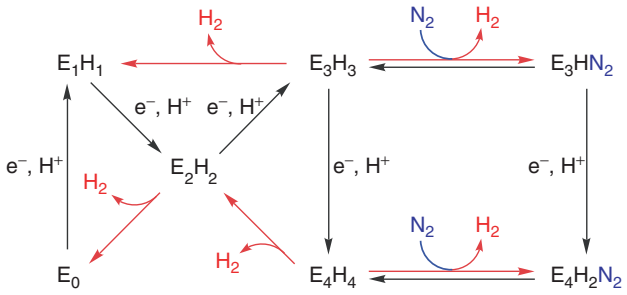


Figure 2. Proposed H_2-evolution pathways in the nitrogenase reaction cycle.[27–31]

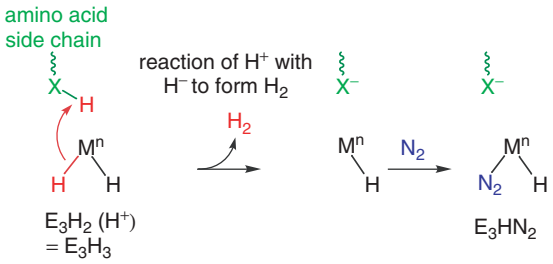
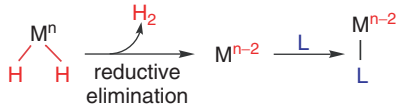


Figure 3. A proposed H_2-evolution from the reaction of dihydride species with a proton bound to an adjacent amino acid side chain.[1,32]

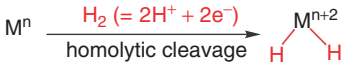
In general, however, it is well known that metal polyhydrides can bind molecules (L) by displacement of H_2 through reductive elimination of H_2:[33–35]



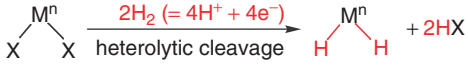
(2)

The oxidation number of the metal ion would be reduced from n to n−2. Thus, the resulting reduced species (M^{n-2}) has a vacant coordination site at which L can be bound and reduced.

Such metal hydrides can be synthesized in the laboratory by using H_2 as a convenient hydrogen source instead of protons with electrons in the reaction cycle of nitrogenases.[36] It is known that homolytic cleavage (oxidative addition) of H_2 is usually promoted by coordinatively unsaturated metal ions whose oxidation number will increase by two to produce metal dihydrides (Eqn. (3)). On the other hand, heterolytic cleavage of H_2 results in the formation of metal dihydrides that involve a net substitution of X^- (typically anionic ligands) by hydride ligands (Eqn. (4)).[37,38] Thus, the oxidation number of the metal ion remains the same. This is an important difference from the homolytic cleavage of H_2. The metal dihydrides prepared by heterolytic cleavage of H_2 are expected to have much stronger reducing ability for reduction of small molecules than the metal dihydrides prepared by homolytic cleavage of H_2.



(3)



(4)

Figure 4 shows possible cycles for the release of two electrons from hydride species prepared by heterolytic cleavage of H_2. The superscripts n and m show the oxidation number of the metal ion (M) and the total charge of the complexes respectively. Two electrons are released from monohydride species without evolution of H_2 (cycle A: $H_2 \rightarrow 2H^+ + 2e^-$), whereas two electrons are released from dihydride species with evolution of H_2 (cycle B: $2H_2 \rightarrow 2H^+ + 2e^- + H_2$). In this context, we have previously reported the first example of formation of a metal polyhydride complex via hetelolytic cleavage of H_2 and subsequent reductive elimination of H_2 from the metal polyhydride complex to generate a reduced metal complex that can reduce nitrate ions (*vide infra*).[39]

PH-DEPENDENT HETEROLYTIC CLEAVAGE OF H_2 AND REDUCTION OF NO_3^- WITH EVOLUTION OF H_2

A water-soluble aqua complex $[Cp^*Ir^{III}(H_2O)_3]^{2+}$ (**1**, Cp^* = η^5-C_5Me_5)[40–47] reacts with three equivalents of H_2 at pH 1–4

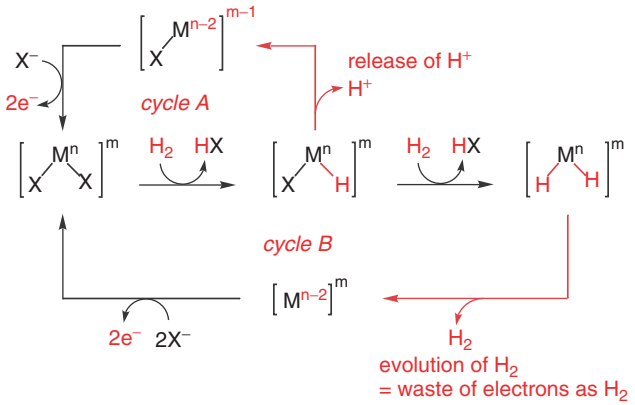
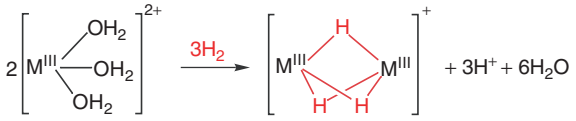


Figure 4. Possible cycles for release of two electrons from monohydride species without evolution of H_2 (cycle A: $H_2 \rightarrow 2H^+ + 2e^-$) or from dihydride species with evolution of H_2 (cycle B: $2H_2 \rightarrow 2H^+ + 2e^- + H_2$).

under ambient conditions (0.1 MPa, 30 °C) to produce a water-soluble dinuclear μ_2-hydride complex $[(Cp^*Ir^{III})_2(\mu_2\text{-}H)_3]^+$ (**2**) through heterolytic cleavage of H_2:



(5)

1 **2**

The structures of **1** and **2** were unequivocally determined by X-ray analysis (Fig. 5).[39,40] It was confirmed by ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS) that the structure of **2** is preserved in the acidic aqueous solution. Consumption of H_2 was measured by a constant-pressure gas burette (Fig. 6). With the progress of the reaction, the pH of the solution is decreased because of the heterolytic cleavage of H_2 giving protons and **2**. To establish the origin of the hydride ligand of **2**, the synthesis of **2** by a reaction of **1** with D_2 has been carried out. ESI-MS results indicate that the deuterium atoms are incorporated in **2**.

Hydrogen atoms bound to transition metals are traditionally called 'hydride ligands', whether or not they display any hydridic behavior. The general description of a bridging

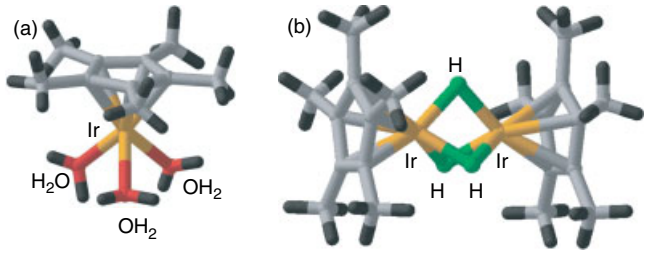


Figure 5. (a) Crystal structure of **1**. (b) Crystal structure of **2** that was obtained from a 0.1 M HNO_3–H_2O (pH 1) solution of **2**.[39,40]

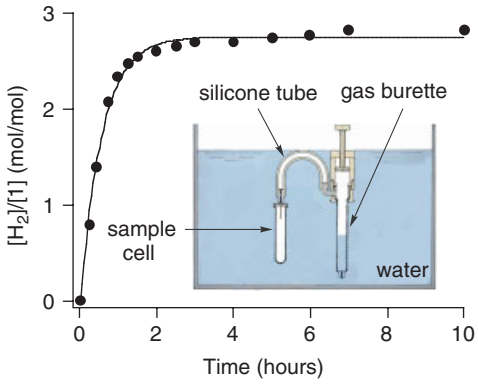
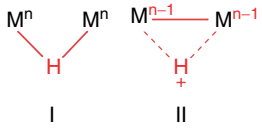


Figure 6. Time course of H_2-consumption on the reaction of **1** (48 mg, 100 μmol) with H_2 (0.1 MPa) in HNO_3–H_2O (10 ml) to yield **2** at pH 2 at 30 °C monitored by a constant-pressure gas burette.[39]

hydride ligand of dinuclear complexes is given in terms of a three-center two-electron bond (represented as **I** in this paper, where M is the metal ion and n is the oxidation number).[48] However, a bridging hydride ligand may also have a protic character when the oxidation number of the metal site is reduced (represented as **II** in this paper).[49–51]



I II

Indeed, in aqueous media, the bridging hydride ligands of **2** display protonic behavior. In the pH range 1 to 3, complex **2** slowly undergoes isotope exchange, Eqn. (6), through $[M\text{–}(H)_2(D)\text{–}M]^+$ and $[M\text{–}(H)(D)_2\text{–}M]^+$ species ($M = Cp^*Ir$).

$$[M\text{–}(H)_3\text{–}M]^+ + 3D^+ \rightleftharpoons [M\text{–}(D)_3\text{–}M]^+ + 3H^+ \quad (6)$$

Figure 7 shows the pH-dependent ^{1}H NMR spectra changes of **2** from pH 1 to −1 at 30 °C. In a pH range of about −0.4 (2.5 M HNO_3–H_2O) to −0.8 (6.3 M HNO_3–H_2O), complex **2** is insoluble because of the common ion effect of NO_3^-. At pH −1 (10 M HNO_3–H_2O), a yellow powder of **2** reacts with HNO_3 to give a pale-yellow solution of **3** with evolution of H_2, nitrogen monoxide (NO), and nitrogen dioxide (NO_2) gases. The structure of **3** was determined by X-ray analysis (Fig. 8) and ESI-MS.

To establish the origin of the H_2 evolved the reaction of **2** with 10 M DNO_3–D_2O was carried out. Intriguingly, gas chromatographic (GC) analyses show that the evolved gas is H_2 not HD or D_2. This clearly indicates that the evolved H_2 is derived from the bridging hydride ligands of **2** rather than from the reaction with D_2O. Below pD 0.4 (pD = pH meter reading + 0.4), the powder of **2** does not undergo H/D exchange with D^+ (DNO_3–D_2O) before the intramolecular

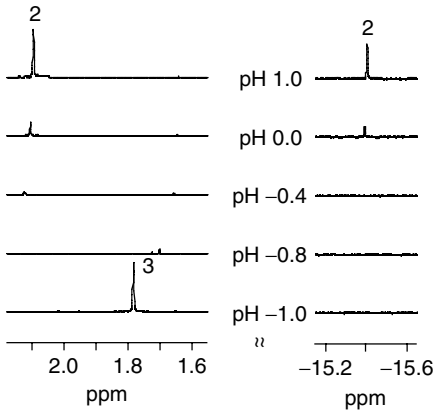


Figure 7. pH-dependent ^{1}H NMR spectral changes of **2** (3.6 mg, 5 μmol) from pH 1 to −1 (10 M HNO_3–H_2O, 1 ml) for 1 h at 30 °C.[39]

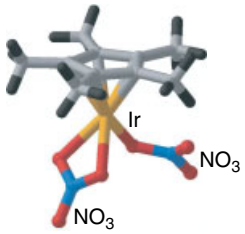
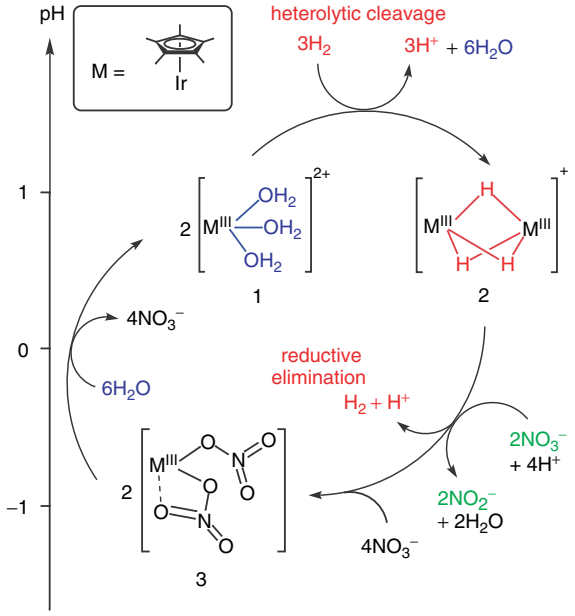


Figure 8. Crystal structure of **3** that was obtained from a 10 M HNO_3–H_2O (pH −1) solution of **3**.[38]

H_2-formation. In contrast, above pD 1.4, complex **2** is soluble in DNO_3–D_2O and undergoes H/D exchange with D^+. It is important to note that the transformation of **2** to **3** does not occur in saturated (*ca.* 10 M) $NaNO_3$–H_2O. These results suggest that, at pH −1, the reductive elimination of H_2 is coupled with the reduction of NO_3^- (Eqn. (7)), i.e. two electrons from **2** are used for reduction of NO_3^-:

$$\overset{(+5)}{NO_3^-} + 2H^+ + 2e^- \rightleftharpoons \overset{(+3)}{NO_2^-} + H_2O \qquad (7)$$

The pH-dependent reaction cycle of heterolytic cleavage of H_2 and reduction of NO_3^- by iridium complexes is summarized in Scheme 1. In the pH range 1 to 4, complex **1** reacts with H_2 to yield **2** as a result of the heterolytic cleavage of H_2. At pH −1, with the evolution of H_2, species **2** provides electrons having the potential to reduce NO_3^- into NO_2^- and is transformed into **3**. To complete the reaction cycle, complex **3** is transformed into **1** by increasing the pH of the solution from −1 to 1. Accordingly, it is possible to repeat the reaction cycle using **1**, H_2, and a pH gradient between pH −1 and pH 1.



Scheme 1.[39]

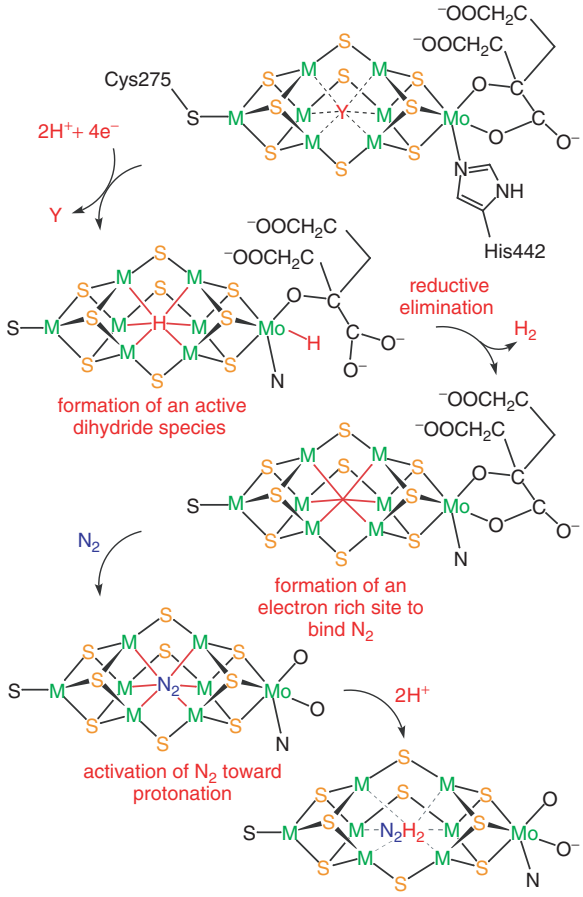


Figure 9. A proposed mechanism for the reduction of N_2 with evolution of H_2 via bridging hydride species by nitrogenases.

A PROPOSED MECHANISM FOR REDUCTION OF N_2 WITH EVOLUTION OF H_2

In the case of nitrogenases, a metal dihydride species, which has the same oxidation number as the starting metal complex, may be formed by a four-electron reduction with two protons rather than a two-electron reduction with two protons. Such active dihydride species may have a bridging hydride ligand at the position of Y and a terminal hydride ligand bound to the molybdenum ion, as shown in Figure 9. Alternatively, two bridging hydride ligands may be formed, as shown in Figure 10. However, the structure of the dihydride species has yet to be clarified.[1,2] In any case, the reductive elimination of H_2 from the dihydride species results in formation of metal complexes in which the oxidation number is reduced by two. Such an electron-rich metal complex, which is coordinatively unsaturated, may have the potential to bind and reduce N_2 with protons, as with the case of **2** in the reduction of NO_3^- in Scheme 1. Thus, we think that the nitrogenases require the evolution of H_2 by reductive elimination from the dihydride species (not by the reaction of the dihydride species with protons bound to an adjacent amino acid side chain) to produce a low valent and coordinatively unsaturated metal site which has the ability to bind and to reduce N_2.

Acknowledgements

Financial support of this research by the Ministry of Education, Science, Sports, and Culture, Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (11228205, 15036242, and 15350033) is gratefully acknowledged. We thank Professor K. Isobe (Kanazawa University), emeritus Professor A. Nakamura (Osaka University), and emeritus Professor Y. Moro-oka (Tokyo Institute of Technology) for valuable discussions.

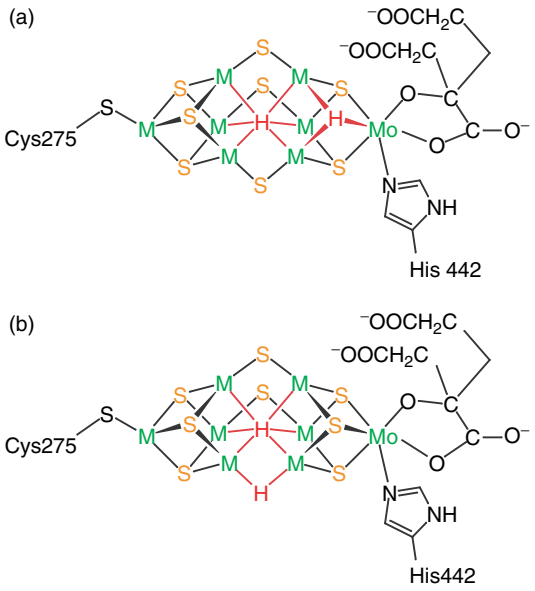


Figure 10. Other possible structures for the active dihydride species.

REFERENCES

1. Leigh GJ. *Nitrogen Fixation at the Millenium*. Elsevier Science: Amsterdam, 2002.
2. Shilov AE. *Metal Complexes in Biomimetic Chemical Reactions*. CRC Press LLC: Boca Raton, 1997; 1–99.
3. Howard JB, Rees DC. *Chem. Rev.* 1996; **96**: 2965.
4. Burgess BK, Lowe DJ. *Chem. Rev.* 1996; **96**: 2983.
5. Eady RR. *Chem. Rev.* 1996; **96**: 3013.
6. Smith BE. *Adv. Inorg. Chem.* 1999; **47**: 159.
7. Christiansen J, Dean DR. Seefeldt LC. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 2001; **52**: 269.
8. Barrière F. *Coord. Chem. Rev.* 2003; **236**: 71.
9. Rees DC, Howard JB. *Curr. Opin. Chem. Biol.* 2000; **4**: 559.
10. Kim J, Rees DC. *Science* 1992; **257**: 1677.
11. Kim J, Rees DC. *Nature* 1992; **360**: 553.
12. Chan MK, Kim J, Rees DC. *Science* 1993; **260**: 792.
13. Kim J, Woo D, Rees DC. *Biochemistry* 1993; **32**: 7104.
14. Mayer SM, Lawson DM, Gormal CA, Roe SM, Smith BE. *J. Mol. Biol.* 1999; **292**: 871.
15. Einsle O, Tezcan FA, Andrade SLA, Schmid B, Yoshida M, Howard JB, Rees DC. *Science* 2002; **297**: 1696.
16. Dance I. *Chem. Commun.* 2002; **3**: 324.
17. Lee HI, Benton PMC, Laryukhin M, Igarashi RY, Dean DR, Seefeldt LC, Hoffman BM. *J. Am. Chem. Soc.* 2003; **125**: 5604.
18. Hinnemann B, Nørskov JK. *J. Am. Chem. Soc.* 2003; **125**: 1466.
19. Lovell T, Liu T, Case DA, Noodleman L. *J. Am. Chem. Soc.* 2003; **125**: 8377.
20. Kozak CM, Mountford P. *Angew. Chem. Int. Ed. Engl.* 2004; **43**: 1186.
21. Seefeldt LC, Dance IG, Dean DR. *Biochemistry* 2004; **43**: 1402.
22. MacKay BA, Fryzuk MD. *Chem. Rev.* 2004; **104**: 385.
23. Rao PV, Holm RH. *Chem. Rev.* 2004; **104**: 527.
24. Lee SC, Holm RH. *Chem. Rev.* 2004; **104**: 1135.
25. Simpson FB, Burris RH. *Science* 1984; **224**: 1095.
26. Alberty RA. *J. Biol. Chem.* 1994; **269**: 7099.
27. Thorneley RNF, Lowe DJ. *Biochem. J.* 1983; **215**: 393.
28. Lowe DJ, Thorneley RNF. *Biochem. J.* 1984; **224**: 877.
29. Thorneley RNF, Lowe DJ. *Biochem. J.* 1984; **224**: 887.
30. Lowe DJ, Thorneley RNF. *Biochem. J.* 1984; **224**: 895.
31. Thorneley RNF, Lowe DJ. *Biochem. J.* 1984; **224**: 903.
32. Henderson RA. Chapter 16 in Peruzzini M, Poli R (Eds). *Recent Advances in Hydride Chemistry*. Elsevier Science: Amsterdam, 2001; 463–505.
33. Crabtree RH. *Inorg. Chim. Acta* 1986; **125**: L7.
34. Tobe ML, Burgess J. *Inorganic Reaction Mechanisms*. Addison Wesley: Longman, Harlow, 1999.
35. Crabtree RH. *The Organometallic Chemistry of the Transition Metals*, 3rd edn. John Wiley: New York, 2001; 149–173.
36. Peruzzini M, Poli R (Eds). *Recent Advances in Hydride Chemistry*. Elsevier Science: Amsterdam, 2001.
37. Brothers PJ. *Prog. Inorg. Chem.* 1981; **28**: 1.
38. Norman AD. *Inorganic Reactions and Methods*. VCH: New York, 1991; 65–79.
39. Ogo S, Nakai H, Watanabe Y. *J. Am. Chem. Soc.* 2002; **124**: 597.
40. Ogo S, Makihara N, Watanabe Y. *Organometallics* 1999; **18**: 5470.
41. Makihara N, Ogo S, Watanabe Y. *Organometallics* 2001; **20**: 497.
42. Ogo S, Makihara N, Kaneko Y, Watanabe Y. *Organometallics* 2001; **20**: 4903.
43. Nakai H, Ogo S, Watanabe Y. *Organometallics* 2002; **21**: 1674.
44. Ogo S, Abura T, Watanabe Y. *Organometallics* 2002; **21**: 2969.
45. Abura T, Ogo S, Watanabe Y, Fukuzumi S. *J. Am. Chem. Soc.* 2003; **125**: 4149.
46. Hayashi H, Ogo S, Abura T, Fukuzumi S. *J. Am. Chem. Soc.* 2003; **125**: 14266.
47. Ogo S, Uehara K, Abura T, Fukuzumi S. *J. Am. Chem. Soc.* 2004; **126**: 3020.

48. Collman JP, Hegedus LS, Norton JR, Finke RG. *Principles and Applications of Organotransition Metal Chemistry*. University Science Books: Mill Valley, CA, 1987; 80–93.
49. Maitlis PM. *Adv. Chem. Ser.* 1979; **173**: 31.
50. Maitlis PM. *Chem. Soc. Rev.* 1981; **10**: 1.
51. Cotton FA, Wilkinson G. *Advanced Inorganic Chemistry*, 4th edn. John Wiley: New York, 1980; 1116–1117.

